Differential expression of human placental growth-hormone variant and chorionic somatomammotropin in culture

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Regulation of human placental growth-hormone variant (hGH-V) in the presence of its own promoter has been studied. At term, 10–20% of placental mRNA is specific for chorionic somatomammotropin (hCS-A and -B) compared with 0.05% hGH-V, yet these genes show more than 90% sequence similarity at the nucleotide level. By using stable gene transfer of intact hGH-V and hCS-A genes into rat pituitary (GC) cells, synthesis and release of hGH-V and hCS are detected. This suggests that hGH-V as well as hCS is secreted during pregnancy. The hCS-A mRNA level is higher than that observed from the hGH-V gene in stably transfected GC cells. Also, a hybrid gene containing hGH-V 5′-flanking DNA was less active than a hybrid hCS-A gene containing equivalent sequences after transient transfection of these cells. This correlates with the binding of a known transcription factor to a proximal region (−97/−66) of the hCS-A promoter, and not the equivalent hGH-V gene sequences. These results indicate that differential expression of hGH-V and hCS in GC cells is related, in part, to the strength of their respective promoters, and suggest a similar mechanism may exist in the placenta.

INTRODUCTION

Until recently the human growth hormone (hGH) family was described as containing pituitary growth hormone (hGH-N), chorionic somatomammotropin (hCS-A or hCS-B) and prolactin [1]. Although additional GH and CS genes were related strongly by their nucleotide sequences, and thus considered part of the hGH gene family [2], evidence of expression *in vivo* was not available. Expression of one of these additional genes, namely that of the placental GH variant (hGH-V), has now been detected, and there is evidence that its gene product might replace hGH-N in directing maternal metabolism during pregnancy [3]. Experiments using transgenic mice expressing the hGH-V protein suggest it has growth-promoting properties in mice [4]. In addition, we have recently shown that hGH-V is lactogenic in a rat lymphoma-Nb2-cell bioassay [5]. The hGH-V gene is expressed in villous tissue of the human placenta [3,6], localizing to the epithelium of the syncytiotrophoblast [7]. This is also the site of hCS-gene expression [8].

Although human placental tumour cells are available, such as JAR and JEG cell lines, and would appear to be good candidates for studying placenta-specific members of the hGH gene family, no expression of endogenous hCS or hGH genes has been observed in recent investigations [9] (see also references cited in [10]). In the absence of human pituitary or placental tumour cells, which express hGH-N or hCS respectively, rat anterior-pituitary (GC) cells have been used to study hCS gene expression and regulation [10–12]. Since the placental hCS-A gene can be expressed and regulated in GC cells, we have pursued the possibility of placental hGH-V expression in these cells after gene transfer. In an earlier attempt, sodium butyrate treatment was used to increase transient transfection efficiency to demonstrate hGH-V promoter activity in GC cells [13]. However, although some expression was detected, levels were not sufficient or the signal consistent enough for examining regulation [13]. In these studies we used stable gene transfer to show that GC cells can be used to examine hGH-V gene expression and regulation. We demonstrate that the hGH-V promoter is weaker than the hCS-A promoter in transfected GC cells. This correlates with an inability of hGH-V proximal promoter DNA to bind a known transcription factor, and suggests a similar mechanism could be responsible, at least in part, for differential expression of hGH-V and hCS in the placenta.

MATERIALS AND METHODS

Plasmid constructions

A plasmid containing hGH-V exon and intron DNA as well as about 496 bp of 5′ and 521 bp of 3′-flanking sequences was cleaved at nucleotide position +1864 with restriction endonuclease *Bgl*II, blunted, and an *Xho*I decanucleotide linker inserted [14]. Thus, if stable transfection of human cells was carried out, the transfected gene could be distinguished from any endogenous gene mRNA. This technique has been used previously to examine transfected and endogenous rat GH mRNAs [15]. Modified hGH-N and hCS-A genes were prepared in a similar manner. Plasmids containing the hGH-N and hCS-A genes were opened at nucleotide positions +1857 and +1428 respectively, and an *Xho*I decanucleotide linker inserted. Hybrid genes with the hCS 5′-flanking (hCSp) and hGH-V 5′-flanking sequences (hGH-2p or hGH-Vp), nucleotides −496 to +1, fused to the bacterial gene coding for chloramphenicol acetyltransferase (CAT), were described elsewhere [13]. The hGH-N gene hybrid was constructed as for hCSp.*cat* and hGH-Vp.*cat* [13] using the hGH-N gene 5′-flanking DNA. The plasmid containing

Abbreviations used: hGH-V, human growth-hormone variant; hCS-A and -B, chorionic somatomammotropin; hGH-N, pituitary growth hormone; CAT (gene *cat*), chloramphenicol acetyltransferase; GC cells, rat anterior-pituitary tumour cells; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal-calf serum; T_3, 3,3′,5-tri-iodothyronine; PBS, phosphate-buffered saline (8 mM-Na_2HPO_4/0.14 M-NaCl, pH 7.3); r.i.a., radioimmunoassay; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate; rGH, rat growth hormone; GHF-1, a transcription factor found in GC cells.

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the Rous-sarcoma-virus promoter fused to the *cat* gene was obtained from Dr. M. Walker, University of California, San Francisco, CA, U.S.A.

To construct a promoterless *cat* gene, the *Sal*I site in pPCAT [16] was converted into a *Hin*dIII site by using linkers. The simian-virus-40 promoter sequences were removed by *Hin*dIII/*Bgl*II digestion, and the plasmid was re-ligated in the presence of *Hin*dIII linkers.

Cell culture and transfections

Rat anterior-pituitary tumour (GC) and human cervical carcinoma (HeLa) cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal-calf serum (FCS) in 100 mm-diameter culture dishes. For preparation of extracts, cells were grown to 80% confluence in eight T175 culture flasks. Stable and transient transfections were done by calcium phosphate–DNA precipitation as described elsewhere [10,13]. Studies with 3,3',5-tri-iodothyronine (T_3) were carried out as described previously [17], with the following modifications. At 6–8 h after the addition of the DNA precipitate, cells were 'shocked' with 20% (v/v) glycerol in DMEM for 2 min, washed twice with phosphate-buffered saline (PBS) and then fed DMEM supplemented with either 2% charcoal-stripped fetal-calf serum (FCS) or 10% serum substitute, 1% charcoal-stripped FCS with or without 10 nM-T_3. The cells were harvested 48 h after gene transfer.

Radioimmunoassay (r.i.a.)

Cells were plated at a density of 5×10^6 cells/100 mm dish in 10% FCS/DMEM. After 24 h the medium was removed, the cells washed with PBS, and fed 5 ml of DMEM supplemented with 25 mM-Hepes. At 18 h, medium was removed from the cells for r.i.a. Solid-phase r.i.a. was done as previously described [17,18], using 1:1000 dilution of hCS antibodies (Dako Corp.) and 33 nCi of iodinated Protein A (sp. radioactivity < 30 mCi/mg)/0.1 ml.

Immunoblotting

Medium, as used for r.i.a. above, was dialysed against 0.1 mM-phenylmethanesulphonyl fluoride, freeze-dried and resuspended in water. The protein concentration was determined by the method of Bradford [19]. Protein samples (20 μg) were fractionated by SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose, and hCS, hGH-N and hGH-V levels were assessed by radiographic detection with hCS antibodies (1:10000; Dako Corp.) and iodinated Protein A [20].

RNA analysis

RNA was isolated by a method adapted from that described by Chomczynski & Sacchi [21]. After the first propan-2-ol precipitation, the RNA pellet was resuspended in 0.4 ml of 10 mM-Tris/HCl (pH 8.0)/1 mM-EDTA, transferred to a Microfuge tube and extracted by using an equal volume of phenol saturated with sterile distilled water, 0.1 vol. of 2 M-sodium acetate, pH 4, and 0.2 vol. of chloroform/3-methylbutan-1-ol mixture (24:1, v/v). The aqueous phase was re-extracted with an equal volume of 3-methylbutan-1-ol and then precipitated with an equal volume of propan-2-ol. Polyadenylated RNA was prepared using oligo(dT)–cellulose chromatography [14]. RNA was denatured with formaldehyde and separated by electrophoresis through a 1.5%-agarose gel [14]. RNA was transferred to nitrocellulose and hybridized with ^{32}P-labelled hGH cDNA and an hGH-V-specific oligonucleotide. The hGH-V-specific oligonucleotide (5'-TATGCCAGCTGGTA-CAGGCGAC-3'), corresponding to codons 18–25 of hGH-V mRNA [22], was prepared by the Regional DNA Synthesis Laboratory at the University of Calgary. Conditions for hybridization were as described previously [22], except the hybridization was done at 65 °C. After hybridization, filters were rinsed with 6 × SSC at room temperature and then washed twice for 15 min with 6 × SSC at room temperature and then washed twice for 15 min with 6 × SSC at 50 °C. Levels of 28 S rRNA were used as a control for RNA loading.

CAT assays

Cells were rinsed with PBS and removed from culture dishes with 1 mM-EDTA in PBS. After centrifugation, the PBS was aspirated and the cells lysed in Tris/HCl/Triton X-100 [0.1 M-Tris/HCl (pH 7.8)/0.1% (v/v) Triton X-100] for 10 min on ice. Insoluble material was removed by centrifugation. A two-phase fluor diffusion assay was used to determine CAT activity [23]. Briefly, cell extract (0.1–0.3 mg) was added to a 7 ml scintillation vial with sufficient Tris/HCl/Triton X-100 to give a total volume of 0.2 ml. The solution was heated to 70 °C for 10 min and cooled to room temperature, and a reaction mix (75 μl), containing 2 μl of [^{3}H]acetyl-CoA (sp. radioactivity about 4 Ci/mmol; 0.5 μCi/assay), 50 μl of 5 mM-chloramphenicol (in water), 7.5 μl of 1 M-Tris/HCl, pH 7.8, and 15.5 μl of water, was added. The reaction mixture was carefully overlaid with 3 ml of organic-phase scintillation cocktail. After 30 min the samples were cycle-counted for radioactivity for at least 1 min for 3–5 h. Quantitative values for CAT activity were determined by regression analysis to give c.p.m./min per mg of cell extract protein.

DNAase 1 footprinting analysis

Nuclear-protein extracts were made from both GC and HeLa cells according to published protocols [24]. Truncated regions of hGH-V or hCS-A 5'-flanking DNA were generated by *Nco*1/*Bam*H1 (−288/+1) and *Pst*1/*Bam*H1 (−278/+1) digestion respectively. The fragments were labelled at the *Bam*H1 end by filling in with Klenow fragment in the presence of ^{32}P-labelled dATP. The labelled DNA (0.5 ng) was incubated without or with nuclear extract (40 μg) and then partially digested with DNAase 1 (Promega). The products were fractionated in a denaturing 7%-acrylamide gel and assessed by autoradiography.

RESULTS

hGH-V and hCS-A protein is released from GC cells stably transfected with intact hGH-V and hCS-A genes

A gene fragment containing hGH-V exon and intron DNA as well as about 496 bp of 5' and 521 bp of 3'-flanking sequences was stably introduced into GC cells (GC[hGH-V] cells). The hGH-N and hCS-A genes were also used to transfect GC cells generating GC[hGH-N] and GC[hCS-A] cells respectively. Pools (greater than 50 clones) were produced and grown to mass. The release of hGH-V into the medium of GC[hGH-V] cells was determined by solid-phase r.i.a. GC cells [$(0.5–1.0) \times 10^7$], transfected or untransfected, were grown in serum-free medium for 18 h. In the absence of specific antibodies to hGH-V, commercially available polyclonal antibodies to hCS (Dako) were used. By using purified hCS and hGH, kindly provided by Ms. H. Cosby and Dr. H. G. Friesen (University of Manitoba) the hCS antibodies used were only slightly less (< 2-fold) specific for pituitary hGH than commercially available hGH antibodies (Dako). Thus, because of the structural similarity between hGH-V and these hormones, common epitopes were expected. In all four determinations, cross-reaction between the hCS antibodies and the medium from transfected relative to untransfected GC cells was seen. This corresponded to about 5 ng (of hCS) per μg of protein released into the medium of GC[hGH-V] cells. In addition, the medium from transfected cells (20 μg of total protein) was fractionated in 12.5%-polyacrylamide gels, trans-

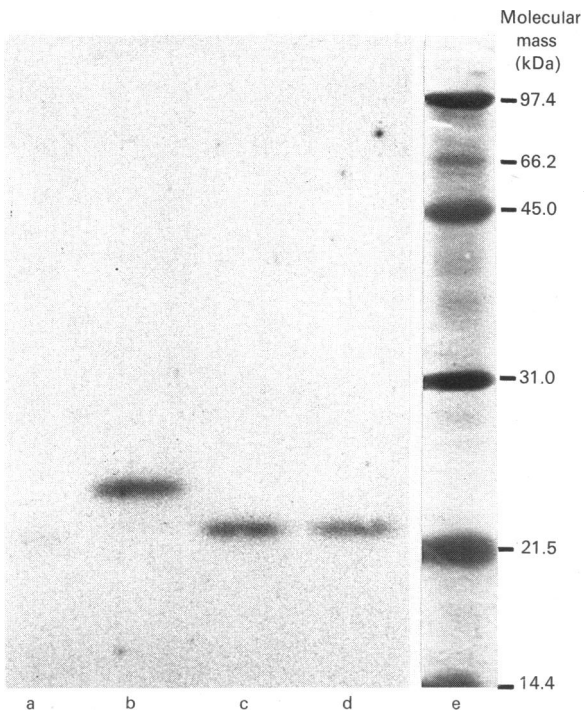

Fig. 1. Release of hGH family members from stably transfected rat pituitary (GC) cells

Proteins (20 μg) from the medium of GC cells non-transfected (a) or transfected with the hCS-A gene (b), hGH-N gene (c) and hGH-V gene (d) were separated by SDS/PAGE, transferred to nitrocellulose and immunostained with anti-hCS antibodies and iodinated Protein A. Lane e shows Coomassie Blue-stained molecular-mass markers (10–100 kDa molecular-mass range; Bio-Rad).

ferred to nitrocellulose, and immunostained with antibodies to hCS and iodinated Protein A (Fig. 1). A single band with a molecular mass of 22 kDa was detected in the medium from GC[hGH-V] cells (lane d). This band is consistently observed above the background seen with medium from untransfected GC cells (lane a). The hGH-V product corresponds in mobility to the predominant product of the hGH-N gene (lane c), but is distinct from the hCS-A gene product (lane b). Detection of an hCS-related protein in the medium of GC[hGH-V] cells indicates that the hGH-V protein can be secreted.

Differential expression of hGH-V and hCS-A mRNA from GC cells stably transfected with intact hGH-V and hCS-A genes

Total cytoplasmic RNA and polyadenylated RNA was isolated from GC, GC[hGH-N], GC[hCS-A] and GC[hGH-V] cells. RNAs were fractionated by electrophoresis, transferred to nitrocellulose membranes and probed with radiolabelled hGH cDNA as well as an oligonucleotide specific for hGH-V. The membrane was washed at high stringency to prevent cross-hybridization to the endogenous rat growth-hormone (rGH) mRNA [10,15,25] and revealed by autoradiography (Fig. 2). A single hGH-V transcript of about 1.0 kb was detected, which corresponds in size to the hGH-N and hCS-A transcripts generated in GC cells [10,15]. The level of hGH-V gene mRNA is lower than that of the hGH-N or hCS-A gene (Fig. 2), suggesting that the hGH-V gene is less active in GC cells.

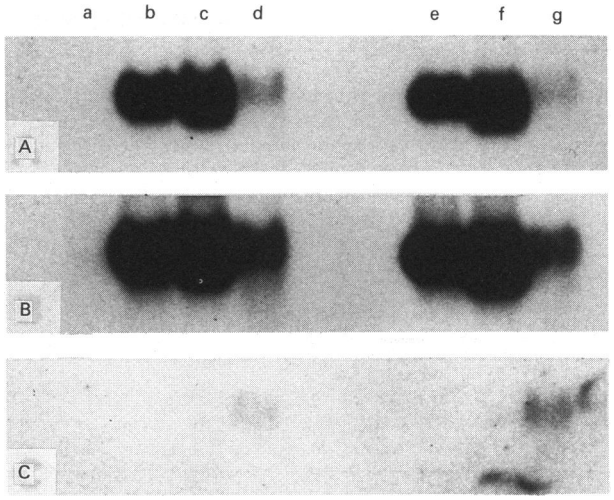

Fig. 2. Expression of stably transfected hGH-gene family members in rat pituitary (GC) cells

A 50 μg portion of total cellular RNA (lanes a–d) as well as polyadenylated RNA isolated from 200 μg of total RNA (lanes e–g) was fractionated by electrophoresis and transferred to nitrocellulose. The nitrocellulose was probed with ^{32}P-labelled hGH cDNA (panels A and B) as well as an hGH-V specific oligonucleotide (panel C). Transcripts were revealed by autoradiography. a, GC cells; b–g, GC cells transfected with hGH-N (b, e), hCS-A (c, f) or hGH-V (d, g). Panels A and B are different exposures of the same hybridization (A, 16 h; B, 96 h).

The 5′-flanking sequences of hGH-V are less active as a promoter than are the 5′-flanking sequences of hCS-A

To compare hGH-V and hCS-A gene promoter activity directly, hybrid genes were made containing nucleotides −496 to +1 from the hGH-V or hCS-A 5′-flanking DNA fused upstream

Table 1. Comparison of hybrid hGH-V and hCS-A gene expression in pituitary (GC) and non-pituitary (HeLa) tumour cells

Hybrid CAT genes containing hGH-V, hCS-A or Rous-sarcoma-virus flanking sequences or no promoter (−p) were used to transfect GC or HeLa tumour cells. CAT activity is expressed as c.p.m./min per mg of cell protein (means ± S.E.M.). The numbers of separate determinations are indicated in parentheses.

Gene	Cells...	CAT activity GC	CAT activity HeLa
RSVp.*cat*		1094.1 ± 123.9 (2)	1830.3 ± 268.3 (2)
Non-transfected		7.4 ± 0.2 (3)	6.0 ± 0.2 (4)
−p.*cat*		15.1 ± 1.1 (6)	8.0 ± 1.0 (3)
hCS-Ap.*cat*		104.3 ± 11.8 (6)	9.0 ± 0.3 (3)
hGH-Vp.*cat*		31.9 ± 1.8 (3)	8.1 ± 1.2 (3)

Table 2. Effect of T_3 on expression of hybrid genes containing members of the hGH gene family in GC cells

GC cells grown in T_3-depleted medium were transfected with hybrid hGH-V, hGH-N, hCS-A or Rous-sarcoma-virus CAT genes. The cells were treated with ($+T_3$) or without ($-T_3$) 10 nM-T_3 and the resulting CAT activity measured. T_3 response is expressed as the ratio of $+T_3$ to $-T_3$ activity. Mean values (±S.E.M.) from at least four determinations are given.

Hybrid gene	T_3 response
hGH-Vp.*cat*	0.8±0.1
hGH-Np.*cat*	1.3±0.4
hCS-Ap.*cat*	3.6±0.8
RSVp.*cat*	0.9±0.1

of the bacterial gene (*cat*) coding for CAT. These hybrid genes were used to transfect transiently GC and non-pituitary, HeLa, cells. CAT activity was determined quantitatively by measuring the rate acetylation of chloramphenicol using a radiolabelled source of acetyl groups. Activity is expressed as c.p.m./min per mg of cell-extract protein used in the assay. The cells were initially transfected with a *cat* gene directed by the Rous-sarcoma-virus promoter (RSVp.*cat*) to demonstrate that the cells are able to take up plasmid DNA and support *cat* gene expression (Table 1). Since it was anticipated that the hGH-V promoter might be weak (see Fig. 2), a promoterless *cat* gene (−p.*cat*) was used in the transfection experiments as a control for any random transcription initiation (Table 1). The hybrid hCS-A and hGH-V genes are both expressed after transient transfection of GC, but not HeLa, cells (Table 1). However, the amount of CAT activity observed relative to that seen with the −p.*cat* gene suggests that the hCS-A promoter is at least 3-fold more active than the hGH-V promoter in GC cells.

We have shown previously that the hCS-A and hGH-N genes respond differently to T_3 treatment in GC cells [10,13]. A hybrid hCS-A gene containing about 500 bp of 5′-flanking DNA was stimulated by T_3 treatment, but a hybrid hGH-N gene with equivalent sequences did not respond to T_3, in spite of about 95% sequence similarity [13]. To determine whether the T_3 response of the hybrid hGH-V gene is more like that of hGH-N or hCS-A, GC cells were grown in T_3-depleted medium for 72 h. These cells were then transiently transfected with hybrid *cat* genes containing nucleotides −496 to +1 of the hGH-V, hCS-A or hGH-N genes. The RSVp.*cat* was used as a control. After transfection, cells were treated with or without 10 nM-T_3 (Table 2). In the presence of T_3 there was an increase in hybrid hCS-A gene activity. However, there was no increase in hybrid hGH-V, hGH-N or RSVp.*cat* gene expression. The T_3 response of hCS-A and lack of response of hGH-V indicates a sufficient sequence variation that, if not important for T_3 regulation *in vivo*, may play a role in the differential expression of these genes in the placenta.

Comparison of transcription factor (GHF-1) binding to hGH-V and hCS-A gene sequences

A transcription factor found in GC cells, commonly referred to as 'GHF-1', binds to a proximal (−92/−65) and a distal (−132/−107) site of the hGH-N 5′-flanking DNA [26]. Binding of GHF-1 to the proximal site is essential for efficient expression in transfected GC cells [12,28]. GHF-1 interacts with equivalent sequences of the hCS-B gene [11]. Although we demonstrated with a functional assay that GHF-1 bound to the proximal

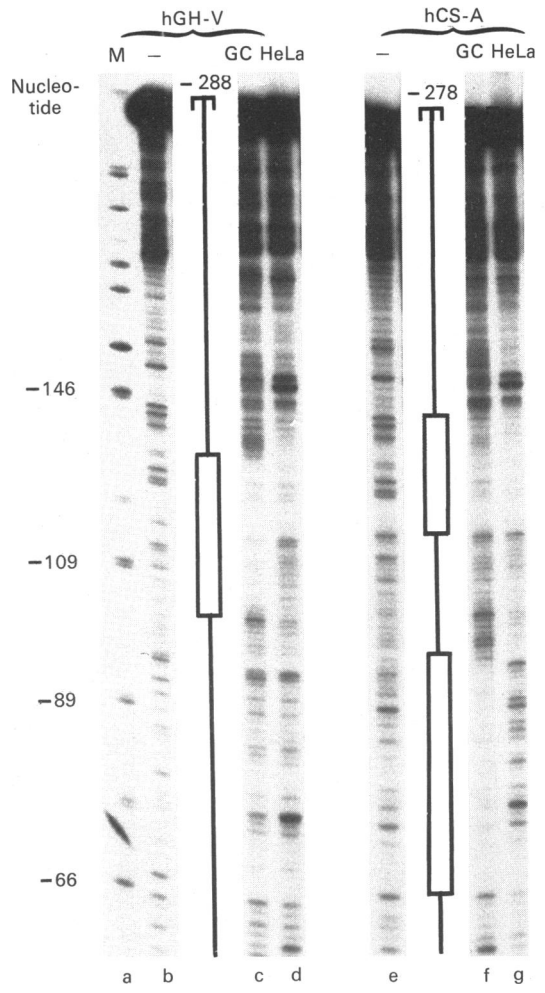

Fig. 3. DNAase 1 protection assay of hGH-V and hCS-A 5′-flanking sequences in the proximal promoter region with nuclear extracts from GC ('GC') and HeLa ('HeLa') cells

Truncated regions of the hGH-V (−288/+1) and hCS-A (−278/+1) genes were generated by *Nco*1/*Bam*H1 and *Pst*1/*Bam*H1 cuts respectively. Fragments were radiolabelled on the strand corresponding to the coding strand. The naked DNA was subjected to a limited ('–') DNAase 1 digest (lanes b and e), or titrated with GC (lanes c and f) or HeLa-cell nuclear protein (lanes d and g) before digestion with DNAase 1. The plasmid, pBR322, cut with *Hpa*II was radiolabelled and used as a marker ('M') in lane a, and the corresponding nucleotide positions are shown. The proximal and distal binding domains identified by the GC-cell extract (lanes c and f) are indicated by boxed domains.

region of the hCS-A gene and was required for efficient expression, no protection assay was done [12]. To demonstrate this binding, and to look for equivalent binding to hGH-V sequences, hGH-V and hCS-A gene fragments containing about 280 bp of 5′-flanking DNA were radiolabelled and then incubated with and without nuclear extracts prepared from GC or HeLa cells. The mixtures were then treated by limited digestion with DNAase 1. The products were resolved by denaturing gel electrophoresis and assessed by autoradiography (Fig. 3). Protection was observed in the distal region of both hGH-V (−140/−107) and hCS-A (−145/−118) 5′-flanking sequences with the GC-cell extract, but only the hCS-A DNA showed protection in the region of the proximal GHF-1 binding domain (−97/−66). In

contrast, with HeLa-cell nuclear extract the hGH-V and hCS-A genes produced a similar DNAase 1 pattern, showing protection only in the region of the distal GHF-1-binding site (Fig. 3).

DISCUSSION

The rat pituitary (GC) cell line stably transfected with the hGH-V gene can synthesize and release hGH-V protein (Figs. 1 and 2). The presence of hGH-V in the medium of these cells indicates that this protein is secreted, suggesting its release from the syncytiotrophoblast during pregnancy. The possibility that hGH-V is also retained by the cell is not ruled out. The modification made in exon 5 of the hGH-V gene used for the present study did not block its release. Further, the ability to detect hGH-V and hGH-N as well as hCS with polyclonal antibodies to hCS indicates, as expected, that these proteins contain common epitopes (Fig. 1).

A transcript is observed from the modified hGH-V gene after stable integration into GC cells (Fig. 2). The 1.0 kb transcript corresponds in size to that obtained with RNA from the hGH-N or hCS-A gene after stable integration in the same cell type [10,15,25], as well as transcripts from the endogenous rGH and hGH genes [15,17]. In placenta, there is evidence for more than one transcript and, thus, product of the hGH-V gene [6,22]. The first transcript corresponds to the transcript observed in the present study. The second transcript, which accounts for a third of the hGH-V mRNA in placenta, is 253 nucleotides larger, corresponding to the inclusion of the fourth intron of the hGH-V gene [6]. Since this larger transcript was not detected, the level of expression might be too low, or alternative splicing necessary to generate the larger transcript is reduced or does not occur with our modified hGH-V gene in GC cells. The location of the modification, 28 nucleotides downstream of the junction between intron 4 and exon 5, is not expected to interfere with splicing. In fact, it does not appear to affect 'correct' splicing, as evidenced by an appropriately sized transcript and detection of released product (Figs. 1 and 2). However, the possibility that it promotes excision of intron 4 and prevents generation of the larger hGH-V2 transcript seen in the placenta [6] cannot be ruled out.

The level of hGH-V mRNA is lower than hCS-A (or hGH-N) mRNA in transfected GC cells (Fig. 2). These results suggest that the hGH-V promoter is not as active as the hCS promoter. This is supported by data obtained when hGH-V and hCS-A promoters were fused to the *cat* gene and CAT activity assessed in GC cells. The hybrid hGH-V gene was at least 3 times less active than the hCS-A gene in transiently transfected GC cells (Table 1). However, these data also suggest that additional mechanisms are required to account for the full difference seen between hGH-V and hCS mRNA levels in GC cells (Fig. 2) and, possibly, in the placenta. Explanations include differences in mRNA stability or a role for additional sequences outside the 5′-flanking DNA tested, which might decrease hGH-V or increase hCS-A promoter activity further. Indeed, Rogers *et al.* [27] identified sequences with enhancer-like activity in the 3′-flanking DNA of the hCS-B gene. However, the hCS-A gene does not possess these sequences, and yet accounts for 6 times more hCS mRNA compared with the hCS-B gene [28]. Alternatively, the difference between the stable- and transient-transfection data might reflect intrinsic variation between the two techniques.

The relatively small number of nucleotide differences between the hGH-V, hCS-A and hGH-N genes allows them to be considered as, to all intents and purposes, point mutations of each other. Consequently, any differences might be responsible for differential regulation. The region containing −92 to −65 of the hGH-N or hCS-A gene is essential for efficient expression in transfected GC cells [12,26,29]. Comparison of the hGH-V and hCS-A sequences in this region reveals six nucleotide differences (see asterisks in Fig. 4), in contrast with the single base change seen between the hGH-N and hCS-A genes (see arrow in Fig. 4). The sequences 5′-CATAAA-3′ are present at nucleotide positions −85 to −80 of the hCS-A gene (Fig. 4) and have been reported to represent an active promoter element [30]. The equivalent sequences in the hGH-V gene are not identical (5′-CCTAAA-3′). We compared the ability of these sequences in the hCS-A and hGH-V gene to bind a transcription factor, GHF-1, from a GC-cell nuclear extract (Fig. 3). Protection of these sequences in the hCS-A gene (−97/−66) was observed as expected, but we were unable to detect binding to the hGH-V 5′-flanking DNA under identical conditions. Since interaction of GHF-1 with this proximal domain is a requirement for efficient hCS-A as well as hGH-N promoter activity in GC cells [12,26], the absence or less effective interaction of a transcription factor at this site in the hGH-V DNA would be expected to result in less gene expression.

We have shown previously that hybrid genes containing hGH-N or hCS-A 5′-flanking sequences respond differently to treatment with T_3 in GC cells. Whereas hybrid hCS-A gene activity is increased [10,13], no increase in hybrid hGH-N gene expression is observed; in fact, slight decreases as well as no effect are

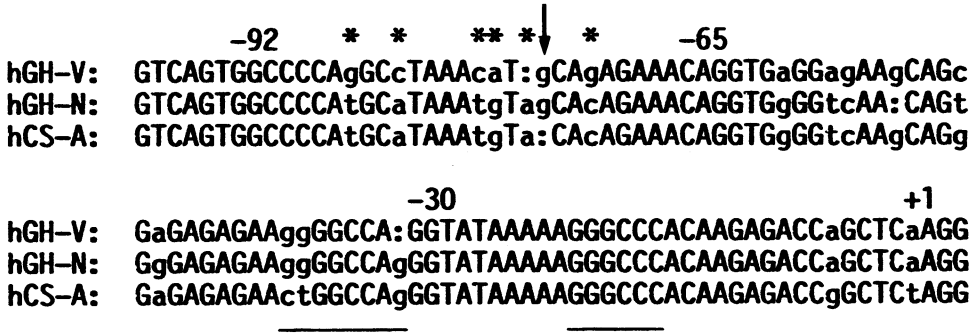

Fig. 4. Nucleotide sequence comparison of the 5′-flanking regions of the hGH-V, hGH-N and hCS-A genes

The numbers indicate the approximate nucleotide positions, owing to an unequal number of insertions/deletions (:). Regions of identical nucleotide sequence are indicated by upper-case letters. The proximal GHF-1-binding region is contained between nucleotides −92 and −65. Variation in the GHF-1-binding region between the hGH-N and hCS-A sequences is marked by an arrow, and that between hGH-V and hCS-A sequences by an asterisk (*). Palindromic sequences are underlined.

indicated in reported data [13,31,32]. Indeed, an increase in hybrid hCS-A gene activity can be observed with only 94 bp of hCS-A gene 5′-flanking sequences (N. L. Eberhardt & P. A. Cattini, unpublished work). We have used the T_3 response to look for sequence variation that, if not important for T_3 regulation *in vivo* [10], may play a role in a differential expression of these genes in placenta. In the present study we show that the hybrid hGH-V gene, like the hybrid hGH-N gene, does not respond positively to T_3 treatment (Table 2). Palindromic sequences have been implicated in regulation of gene expression [33] and are certainly potential sites of specific recognition and factor interaction, as evidenced with many restriction endonucleases. Sequences in the region −94 to +1 of the hCS-A gene were compared with those from the non-responsive hGH-V and hGH-N genes. This reveals an 8 bp palindromic domain 5′-CTGGCCAG-3′ present only in the hCS-A gene at nucleotide positions −40 to −33, immediately upstream of the 'TATA' box, and a 6 bp palindrome, 5′-GGGCCC-3′, at nucleotide positions −22 to −17, present in all three genes (underlined in Fig. 4). Thus the sequences −40 to −33 could play a role in regulation of the hCS-A gene and might also contribute to the differential level of hCS-A and hGH-V gene expression.

At term, 10–20% of placental polyadenylated RNA is hCS mRNA [34] relative to 0.05% for hGH-V mRNA [6]. This indicates a lower level of hGH-V gene expression as compared with the hCS-A and hCS-B genes in the syncytiotrophoblast [35,36]. Since these genes share more than 90% nucleotide sequence similarity in their structural and flanking DNA [1], the mechanism by which these genes are regulated in the same cell during pregnancy is particularly intriguing. Our data suggest the relative strengths of the hGH-V and hCS-A promoters may play some part in the differential level of expression of these two related genes that is seen in the same cell at the same developmental stage.

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